

A Homozygous Nonsense Mutation (428G→A) in the Human Secretor (*FUT2*) Gene Provides Resistance to Symptomatic Norovirus (GGII) Infections

Maria Thorven,^{1†} Ammi Grahn,^{2†} Kjell-Olof Hedlund,³ Hugo Johansson,⁴ Christer Wahlfrid,⁵ Göran Larson,² and Lennart Svensson^{6*}

Departments of Virology¹ and Bacteriology,³ Swedish Institute for Infectious Disease Control, Solna, Department of Clinical Chemistry, and Transfusion Medicine, Sahlgrenska University Hospital, Göteborg,² Hospital Infection Control Unit, Clinical Microbiology and Immunology,⁴ Department of Medicine,⁵ University of Lund, Lund, and Division of Molecular Virology, Medical Faculty, University of Linköping,⁶ Sweden

Received 10 May 2005/Accepted 21 September 2005

Noroviruses (formerly Norwalk-like viruses) are a major cause of acute gastroenteritis worldwide and are associated with a significant number of nosocomial and food-borne outbreaks. In this study we show that the human secretor *FUT2* gene, which codes for an $\alpha(1,2)$ -fucosyltransferase synthesizing the H-type 1 antigen in saliva and mucosa, is associated with susceptibility to norovirus infections. Allelic polymorphism characterization at nucleotide 428 for symptomatic ($n = 53$) and asymptomatic ($n = 62$) individuals associated with nosocomial and sporadic norovirus outbreaks revealed that homozygous nonsense mutation (428G→A) in *FUT2* segregated with complete resistance for the disease. Of all symptomatic individuals, 49% were homozygous (SeSe) and 51% heterozygous (Sese⁴²⁸) secretors, and none were secretor negative (se⁴²⁸se⁴²⁸), in contrast to 20% nonsecretors (se⁴²⁸se⁴²⁸) among Swedish blood donors ($n = 104$) ($P < 0.0002$) and 29% for asymptomatic individuals associated with nosocomial outbreaks ($P < 0.00001$). Furthermore, saliva from secretor-positive and symptomatic patients but not from secretor-negative and asymptomatic individuals bound the norovirus strain responsible for that particular outbreak. This is the first report showing that the *FUT2* nonsecretor (se⁴²⁸se⁴²⁸) genotype is associated with resistance to nosocomial and sporadic outbreaks with norovirus.

Noroviruses are the major cause of acute gastroenteritis worldwide among adults and are associated with the illness “winter vomiting disease,” characterized by a short incubation period (24 to 48 h) and significant vomiting and diarrhea. While the viruses are highly contagious, with attack rates up to 70% (12), volunteer challenge studies have shown that a subset of individuals remain uninfected even after repeated challenges (2, 20, 26). At present, it is not clear why a fraction of individuals remain uninfected in norovirus outbreaks and why some volunteers are repeatedly resistant to experimental Norwalk virus inoculations. Recently, it was suggested that histo-blood group antigens and the secretor status might be associated with experimental Norwalk virus infections (4, 10, 11, 16, 17).

The *FUT2* gene, which is responsible for the secretor phenotype, encodes an $\alpha(1,2)$ -fucosyltransferase that regulates the expression of the ABH antigens in saliva and mucosal tissues and secretions. The *FUT2* gene has a significant polymorphism with typical ethnic specificity (13). The nonsense mutation 428G→A (Trp143→stop) is characteristic for the dominating nonsecretor allele (se⁴²⁸) in Europeans and appears in about 20% of the Caucasian population (13).

The facts that about 20% of Europeans are nonsecretors and norovirus attack rates seldom exceeds 80% in a given

outbreak led us to investigate, throughout a series of prospective studies, if the *FUT2* secretor gene was associated with resistance to nosocomial and sporadic outbreaks caused by genogroup II (GGII) noroviruses, which dominate in all parts of the world (14).

MATERIALS AND METHODS

Subjects and samples. The study included 115 saliva samples, of which 53 were collected from norovirus-infected and symptomatic individuals and 62 from asymptomatic individuals.

Case definitions. A patient with gastroenteritis was defined as a patient with vomiting (one or more times in 24 h) and/or diarrhea (more than two watery stools in 24 h).

Electron microscopy. All fecal samples were screened for viruses by electron microscopy (EM) as described previously (6).

RNA extraction. RNA was extracted from 100 μ l stool suspension by use of the guanidinium thiocyanate-silica extraction method (1).

Norovirus RT-PCR. Specimens were investigated for the presence of Norwalk-like human calicivirus by a reverse transcription-PCR (RT-PCR) as previously described by Vinjé et al. (23, 24). Briefly, RNA was extracted from 50 μ l of a 10% stool suspension using the guanidinium thiocyanate-silica extraction method (1). RT was performed as follows. Five microliters of extracted RNA was annealed with 50 pmol JV 13 (5'-TCA TCA CCA TAG AAA GAG) in a total volume of 9 μ l and then added to a reaction mix containing 10 mM Tris, pH 8.3, 50 mM KCl, 3 mM MgCl₂, 1 mM deoxynucleoside triphosphate, and 100 units of Moloney murine leukemia virus RT (Superscript; Life Technologies). Reaction mixtures were incubated for 1 h at 42°C. Five microliters of the RT reaction was added to a PCR mix composed of 10 mM Tris, pH 9.2, 75 mM KCl, 1.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphate, 15 pmol JV12 (5'-ATA CCA CTA TGA TGC AGA TTA), and 2.5 U *Taq* polymerase. Forty reaction cycles were carried out with 1 min at 94°C, 1.5 min at 37°C, and 1 min at 74°C followed by a final incubation at 74°C for 7 min. One-fifth of the reaction volume was

* Corresponding author. Mailing address: Division of Molecular Virology, Medical Faculty (IMK), University of Linköping, 581 85 Linköping, Sweden. Phone: 46 13 228803. Fax: 46 13 224789. E-mail: lensv@imk.liu.se.

† These two authors contributed equally to this work.

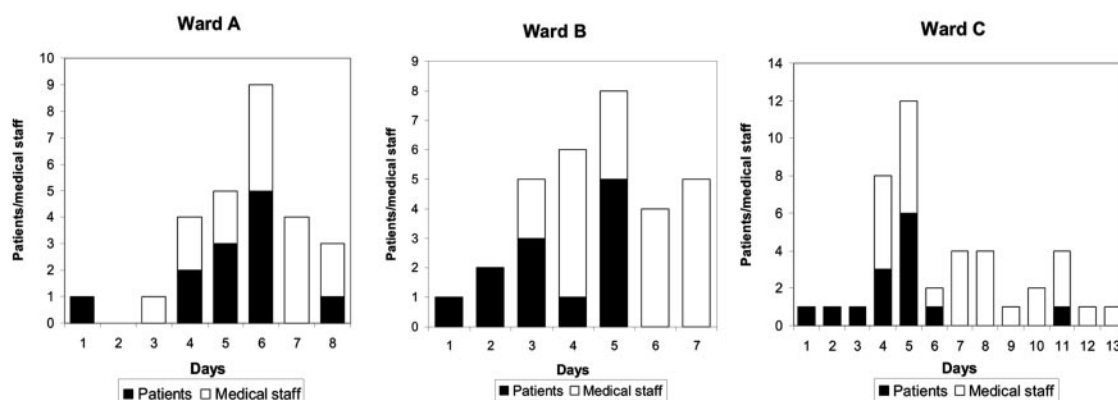


FIG. 1. Time course of the nosocomial outbreaks. Ward A included 15 medical staff and 12 patients with symptomatic norovirus infection; ward B included 19 medical staff and 12 patients; ward C included 28 medical staff and 14 patients.

analyzed on agarose gels. A positive and specific reaction resulted in a 326-bp product, located in the gene for the RNA-dependent RNA polymerase.

FUT2 gene polymorphism characterization. DNA was extracted from saliva samples, and the *FUT2* (secretor) genotype was determined using sequence-specific primers and PCR (PCR-SSP) as described previously (3, 21). The same antisense primer (5'-GGCTGCCTCTGGCTTAAAG) was used in all PCR amplifications but was paired with the sense primers for *FUT2* wild-type 385A (5'-AGGAGGAATACCGCCACAT), mutated 385T (5'-GAGGAGGAATACCGCCACT), wild-type 428G (5'-GCTACCCCTGCTCCTGG), mutated 428A (5'-CGGCTACCCCTGCTCCTA), wild-type 571C (5'-TAGGGTCCATGTTTCGCC), and mutated 571T (5'-GTAGGGGTCCATGTTTCGCT), giving fragments of 574-, 575-, 530-, 532-, 388-, and 390-bp lengths. The human growth hormone gene was used as an internal PCR control, giving a 428-bp product.

Binding of norovirus to saliva. Saliva samples were boiled, centrifuged at $10,000 \times g$ for 5 min, and diluted 1:500 in enzyme-linked immunosorbent assay (ELISA) coating buffer (0.1 M carbonate-bicarbonate buffer, pH 9.6) essentially as described previously (4, 17). After 2 h of incubation at 37°C followed by overnight incubation at 4°C, the plates were washed three times with washing buffer and blocked (bovine serum albumin [3%]-phosphate-buffered saline) for 60 min at 37°C followed by three more washes. Stool sample suspensions (10%) were diluted 1:2 in phosphate-buffered saline-Tween 20 (0.05%-bovine serum albumin (0.5%)) and incubated for 2 h at 37°C followed by three washes and incubation with peroxidase-labeled genogroup I and II-specific norovirus polyclonal antibody (DAKO, Denmark) for 1 h at 37°C. The reaction was developed using 3',3',5',5'-tetramethylbenzidine (ICN Biochemicals).

Nucleotide sequencing. Norovirus-specific PCR products were sequenced using the BigDye Terminator cycle sequencing kit (Perkin-Elmer) on an automated ABI PRISM model 3100 machine. Sequence analysis, alignments, and phylogenetic comparisons were done using the Lasergene software package (DNASTAR, Inc., Madison, Wis.).

Genotyping of norovirus. All norovirus-positive samples were genotyped by reverse line blot hybridization as described previously (12, 25).

Statistical methods. Fisher's exact test (two-sided) was used to test significance of differences in distribution of secretor-positive (SeSe or Sese⁴²⁸) and secretor-negative (se⁴²⁸se⁴²⁸) individuals among symptomatic, asymptomatic, and control individuals.

Ethical approval. The study was approved by local ethical committees at the involved universities and included informed consent for genetic testing of saliva samples.

RESULTS AND DISCUSSION

Information about the secretor status in the Swedish population was obtained from 104 unselected plasma donors genotyped by PCR-restriction fragment length polymorphism for the *FUT2* gene 428G→A nonsense mutation (8, 15). A majority, 54%, were heterozygous secretors (Sese⁴²⁸), 26% homozygous secretors (SeSe), and 20% homozygous nonsecretors

(se⁴²⁸se⁴²⁸), consistent with previous reports that about 20% of Europeans are genetically nonsecretors.

On 20 September 2002, a patient with gastroenteritis was transferred to an internal medicine ward (ward A). Two days later, an assistant nurse in the ward fell ill with acute vomiting and diarrhea. On the following 5 days, 14 additional medical staff and 11 patients on the ward got sick with gastroenteritis. Thus, all together, 15 medical staff and 12 patients had gastroenteritis within 8 days (Fig. 1, Ward A). The symptoms and the outbreak were in agreement with a norovirus infection (i.e., acute onset of gastroenteritis that lasts for a few days) (12). A stool sample taken from one symptomatic patient contained norovirus as determined by electron microscopy and norovirus-specific PCR (12). Genotyping revealed that the virus belonged to the genogroup II cluster and was Lordsdale-like (LDV), a genotype most commonly found in nosocomial outbreaks in Europe (14).

Saliva samples were obtained from 41 of the medical staff and from 19 of the patients, including 10 of the 12 patients and 13 of the 15 medical staff with gastroenteritis. DNA was obtained from 50 of the 60 saliva samples and examined for mutations partially or completely inactivating *FUT2* (385A→T, 571C→T, and 428G→A) by PCR-SSP, a technique previously used for Lewis secretor and ABH genotyping (3, 21) (Fig. 2). Of the symptomatic individuals from this outbreak, 47% were homozygous secretors (SeSe), 53% heterozygous secretors (Sese⁴²⁸), and most interestingly, none were secretor negative (Fig. 3), in contrast to 19% (se⁴²⁸se⁴²⁸) who either were not exposed or were resistant to infection. Three of the six nonsecretors were medical staff (ages 39, 45, and 59), and three were patients (ages 88, 89, and 89). Interestingly, two of the three secretor-negative patients were nursed together in the same rooms as symptomatic patients with gastroenteritis, of which one was the index patient. In spite of the potential exposure from a symptomatic roommate, none of these two secretor-negative patients had any clinical symptoms of gastroenteritis. To investigate if the outbreak strain could bind to saliva from infected individuals, a saliva-based ELISA was established. As shown in Fig. 4, saliva from secretor-positive and ill individuals but not from secretor-negative individuals bound the LDV associated with the outbreak (LDV, Ward A).

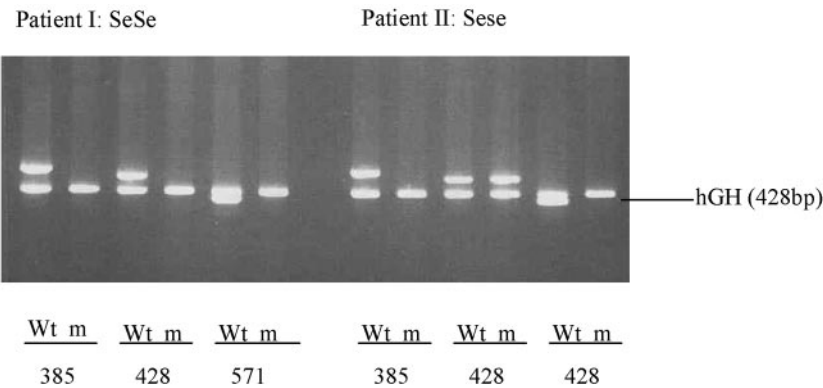


FIG. 2. Example of *FUT2* gene polymorphism characterization. The PCR-SSP patterns from two patients identify their secretor or nonsecretor genotype. Genotypes were characterized by detecting the presence or absence of PCR products indicating wild-type (Wt) or mutated (m) alleles at nucleotides 385, 428, and 571. Human growth hormone (hGH), present in all lanes, served as an internal PCR control. PCR products were separated on 1% agarose gels and detected by ethidium bromide staining. Patient I is a SeSe homozygous secretor. Patient II is a Sese heterozygous secretor.

The second nosocomial outbreak with norovirus occurred in a pediatric ward (Fig. 1, Ward B) on 22 November 2002 with an index patient with acute gastroenteritis. Within the following 6 days, 12 patients and 19 medical staff fell ill with gastroenteritis (Fig. 1, Ward B). In fecal samples collected from ill patients, norovirus were identified by electron microscopy and PCR for three patients. Genotyping by reversed line blotting (25) revealed that all viruses again belonged to genogroup II and were Lordsdale-like. Saliva samples were collected from 38 of the medical staff, of which 12 had symptoms. Unfortunately, saliva could not be collected from patients. Of the 38 saliva samples investigated, secretor status could be established in 28. None of the 7 symptomatic medical staff was secretor negative, in contrast to 43% (9/21) secretor-negatives among the medical staff that remained without clinical symptoms (Fig. 3).

A third nosocomial outbreak affected orthopedic patients (Fig. 1, Ward C) and started on 8 December 2002 with the introduction of a patient with gastroenteritis. One day later, another patient in the ward had similar symptoms, and there-

after, patients and medical staff fell ill during the following 10 days. In all, 14 patients and 28 medical staff experienced gastroenteritis. The epidemic and symptoms were in agreement with norovirus infection. Norovirus was also confirmed by PCR in stool samples from four patients. Once again the norovirus identified belonged to genogroup II and the Lordsdale-like cluster. Saliva samples were obtained from 26 of the medical staff, of which 16 had gastroenteritis. Saliva samples could not be collected from patients. Secretor status was established from 18/26 salivas, and none of the 12 symptomatic individuals was a nonsecretor, in contrast to 50% (3/6) among those who remained asymptomatic. To better understand the phenotype correlation between secretor status and resistance to norovirus infection, saliva samples from secretor and nonsecretor individuals were tested in a saliva ELISA. As shown in Fig. 4, saliva from ill secretor individuals but not from nonsecretors recognized the virus strain responsible for the outbreak, strongly

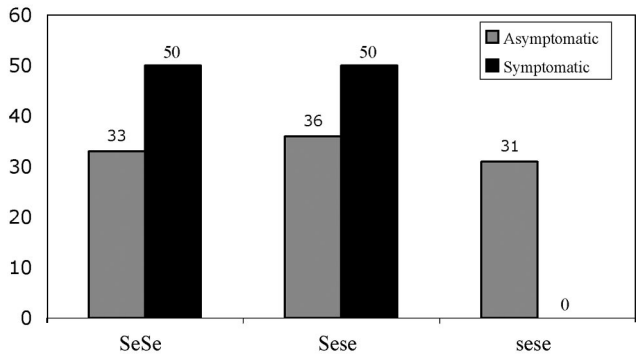


FIG. 3. Allelic distribution of the nonsense mutation (428G→A) in the *FUT2* gene and resistance to symptomatic nosocomial (wards A to C) norovirus infections caused by genogroup II Lordsdale-like strains. A total of 38 saliva samples from symptomatic patients and 58 saliva samples from asymptomatic individuals were investigated. Homozygous secretors, SeSe; heterozygous secretors, Sese; nonsecretors, sese.

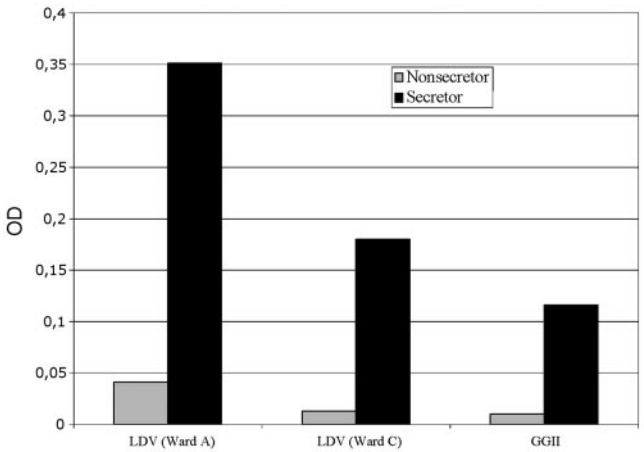


FIG. 4. Secretor-positive salivas from symptomatic individuals recognize the outbreak virus strain by ELISA. LDV (Ward A), virus and saliva from outbreak A; LDV (Ward C), virus and saliva from outbreak C; GGII, virus from a sporadic outbreak, with Lordsdale-like virus with saliva from outbreak A.

suggesting that receptors for norovirus GGII are present in saliva from secretors but not from nonsecretors.

To obtain further information about the role of *FUT2* in norovirus infections, fecal and saliva samples were collected from community outbreaks. Of salivas collected from 19 individuals associated with 3 community outbreaks with noroviruses belonging to genotypes GGII/Melksam, GGI/Sindleshm, and another GGII strain, 4 individuals were asymptomatic and 15 symptomatic with vomiting and diarrhea. Of the asymptomatic individuals, two were Sese⁴²⁸ and two SeSe, and of the 15 symptomatic individuals, 47% (7/15) were SeSe and 53% (8/15) Sese⁴²⁸. Thus, none of the 15 symptomatic individuals was a nonsecretor.

Disease studies have given different results on whether individual ABO (H) blood groups are related to an increased risk of norovirus infection or not. Hutson et al. (10) first reported that individuals with the O phenotype were more likely to be infected with Norwalk virus belonging to genogroup I viruses, whereas individuals with an A or B histo-blood group antigen had a decreased risk of infection. A similar observation was made by Hennessy et al. (7) in investigating a norovirus outbreak at a British military field hospital, finding a reduced susceptibility of blood group B individuals to symptomatic norovirus infections. These observations are in contrast to those of Meyer et al. (18), who reported that persons with the O phenotype were significantly less affected than expected from the normal distribution. Furthermore, Rockx et al. (22) recently reported that individuals secreting type B antigens in saliva were significantly protected against infection with genogroup I virus (2 of 22 individuals) and were also less likely to acquire norovirus-specific immunoglobulin G.

Possible explanations for these different observations may be that the secretor status was not determined in all cases and, secondly, whether it was determined by phenotyping rather than by genotyping (3, 19, 21). Norwalk virus have been found to bind to gastrointestinal cells in vitro independently of ABO (H) blood groups but with dependence on secretor status (17) and found to correlate to resistance against genogroup I viruses (16), suggesting that the *FUT2* gene and secretor status are susceptibility markers for Norwalk virus infection. The fact that nonsecretors do not express blood group A or B in saliva or other body fluids could suggest a coincidental correlation between the ABO phenotype in blood and infection of the intestinal mucosa. Indeed, individuals can have the A or B phenotype in the blood, but if secretor negative, they do not express the A or B antigen in saliva or mucosa. However, it cannot be ruled out that certain noroviruses are secretor independent, since multiple receptor specificities have been reported from in vitro studies (5, 9).

Our studies, the first to include a host genetic approach and to examine secretor status for nosocomial and sporadic outbreaks, revealed several novel observations. We report for the first time that a nonsense mutation (428G→A) in the *FUT2* gene is strongly associated ($P < 0.00001$) with resistance to winter vomiting disease. In fact, all (100%) individuals with a confirmed norovirus infection studied have so far been secretor positive (Table 1).

Furthermore, we also show that only homozygous nonsecretors (se⁴²⁸se⁴²⁸) are protected. The inactivating mutation examined in this study is by far the most common in Europe and

TABLE 1. Association between a *FUT2* nonsense mutation (428G→A) and resistance to symptomatic nosocomial and sporadic norovirus infections in Sweden

Secretor status	No. (%) of subjects with secretor status		
	Blood donors in Sweden	Asymptomatic individuals	Symptomatic individuals
Secretors			
SeSe	27 (26)	21 (34)	26 (49)
Sese ⁴²⁸	56 (54)	23 (36)	27 (51)
Nonsecretors			
se ⁴²⁸ se ⁴²⁸	21 (20)	18 (29)	0 (0) ^{a,b}
Total ($n = 219$)	104	62 ^c	53 ^c

^a Fisher's exact test (two-sided), symptomatic individuals versus blood donors ($P < 0.0002$).

^b Fisher's exact test (two-sided), symptomatic individuals versus asymptomatic individuals ($P < 0.00001$).

^c Includes both nosocomial and sporadic outbreaks.

was found to occur homozygously in 20% among Swedish plasma donors (Table 1). However, it should be mentioned that protective immunity might very well play a role, although correlates of protection remains to be identified.

In vitro studies have shown that binding of Norwalk-like viruses to ABO, Lewis, and secretor histo-blood group antigens are strain specific and that different attachment mechanisms exist (4, 9). Since cells transfected with the *FUT2* gene enhance norovirus binding to nonpermissive cells (17), it is reasonable to believe that H type 1 or related blood group antigens act as receptors for norovirus. Our study supports this hypothesis, and we observed that the saliva ELISA by Marionneau et al. (17) was successfully applied to show that the virus causing disease also could be recognized by the saliva of the patient. This new observation, together with our secretor polymorphism data, provide biochemical and genetic explanations for resistance to noroviruses belonging to the common GGII Lordsdale-like cluster.

ACKNOWLEDGMENTS

This study was supported by the Swedish Research Council (grant 8266), the European Union (grants QLRT-1999-00634 and QLRT-1999-00594), and the Health Research Council of Southeast Sweden.

We thank Gustaf Rydell for statistical calculations, Ann-Christin Hammarlund at the Hospital Infection Control Unit, and the medical staff and patients at the departments of Medicine, Orthopaedics, and Pediatrics, Lund University Hospital, Lund, who participated in the studies.

REFERENCES

1. Boom, R., C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen, and J. van der Noordaa. 1990. Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* **28**:495–503.
2. Gary, G. W., L. J. Anderson, B. H. Keswick, P. C. Johnson, H. L. DuPont, S. E. Stine, and A. V. Bartlett. 1987. Norwalk virus antigen and antibody response in an adult volunteer study. *J. Clin. Microbiol.* **25**:2001–2003.
3. Grahn, A., A. Elmgren, L. Aberg, L. Svensson, P. A. Jansson, P. Lonnroth, and G. Larson. 2001. Determination of Lewis FUT3 gene mutations by PCR using sequence-specific primers enables efficient genotyping of clinical samples. *Hum. Mutat.* **18**:358–359.
4. Harrington, P. R., L. Lindesmith, B. Yount, C. L. Moe, and R. S. Baric. 2002. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. *J. Virol.* **76**:12335–12343.
5. Harrington, P. R., J. Vinje, C. L. Moe, and R. S. Baric. 2004. Norovirus capture with histo-blood group antigens reveals novel virus-ligand interactions. *J. Virol.* **78**:3035–3045.

6. Hedlund, K. O., E. Rubilar-Abreu, and L. Svensson. 2000. Epidemiology of calicivirus infections in Sweden, 1994–1998. *J. Infect. Dis.* **181**(Suppl. 2): S275–S280.
7. Hennessy, E. P., A. D. Green, M. P. Connor, R. Darby, and P. MacDonald. 2003. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J. Infect. Dis.* **188**:176–177.
8. Henry, S., R. Mollicone, J. B. Lowe, B. Samuelsson, and G. Larson. 1996. A second nonsecretor allele of the blood group alpha(1,2)fucosyl-transferase gene (FUT2). *Vox Sang.* **70**:21–25.
9. Huang, P., T. Farkas, S. Marionneau, W. Zhong, N. Ruvoen-Clouet, A. L. Morrow, M. Altaye, L. K. Pickering, D. S. Newburg, J. LePend, and X. Jiang. 2003. Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. *J. Infect. Dis.* **188**:19–31.
10. Hutson, A. M., R. L. Atmar, D. Y. Graham, and M. K. Estes. 2002. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J. Infect. Dis.* **185**:1335–1337.
11. Hutson, A. M., R. L. Atmar, D. M. Marcus, and M. K. Estes. 2003. Norwalk virus-like particle hemagglutination by binding to H histo-blood group antigens. *J. Virol.* **77**:405–415.
12. Johansson, P. J., M. Torven, A. C. Hammarlund, U. Bjorne, K. O. Hedlund, and L. Svensson. 2002. Food-borne outbreak of gastroenteritis associated with genogroup I calicivirus. *J. Clin. Microbiol.* **40**:794–798.
13. Koda, Y., H. Tachida, H. Pang, Y. Liu, M. Soejima, A. A. Ghaderi, O. Takenaka, and H. Kimura. 2001. Contrasting patterns of polymorphisms at the ABO-secretor gene (FUT2) and plasma alpha(1,3)fucosyltransferase gene (FUT6) in human populations. *Genetics* **158**:747–756.
14. Koopmans, M., E. van Strien, and H. Vennema. 2003. Molecular epidemiology of human caliciviruses, p. 523–533. *In* U. Desselberger and J. Gray (ed.), *Viral gastroenteritis*, vol. 9. Elsevier, Amsterdam, The Netherlands.
15. Larson, G., L. Svensson, L. Hynsjo, A. Elmgren, and L. Rydberg. 1999. Typing for the human Lewis blood group system by quantitative fluorescence-activated flow cytometry: large differences in antigen presentation on erythrocytes between A(1), A(2), B, O phenotypes. *Vox Sang.* **77**:227–236.
16. Lindesmith, L., C. Moe, S. Marionneau, N. Ruvoen, X. Jiang, L. Lindblad, P. Stewart, J. LePend, and R. Baric. 2003. Human susceptibility and resistance to Norwalk virus infection. *Nat. Med.* **9**:548–553.
17. Marionneau, S., N. Ruvoen, B. Le Moullac-Vaidye, M. Clement, A. Cailleau-Thomas, G. Ruiz-Palacios, P. Huang, X. Jiang, and J. Le Pendu. 2002. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* **122**:1967–1977.
18. Meyer, E., W. Ebner, R. Scholz, M. Dettenkofer, and F. D. Daschner. 2004. Nosocomial outbreak of norovirus gastroenteritis and investigation of ABO histo-blood group type in infected staff and patients. *J. Hosp. Infect.* **56**: 64–66.
19. Nilsson, M., K. O. Hedlund, M. Thorhagen, G. Larson, K. Johansen, A. Ekspong, and L. Svensson. 2003. Evolution of human calicivirus RNA in vivo: accumulation of mutations in the protruding P2 domain of the capsid leads to structural changes and possibly a new phenotype. *J. Virol.* **77**:13117–13124.
20. Parrino, T. A., D. S. Schreiber, J. S. Trier, A. Z. Kapikian, and N. R. Blacklow. 1977. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N. Engl. J. Med.* **297**:86–89.
21. Procter, J., J. Crawford, M. Bunce, and K. I. Welsh. 1997. A rapid molecular method (polymerase chain reaction with sequence-specific primers) to genotype for ABO blood group and secretor status and its potential for organ transplants. *Tissue Antigens* **50**:475–483.
22. Rockx, B. H., H. Vennema, C. J. Hoebe, E. Duizer, and M. P. Koopmans. 2005. Association of histo-blood group antigens and susceptibility to norovirus infections. *J. Infect. Dis.* **191**:749–754.
23. Vinje, J., S. Altena, and M. Koopmans. 1997. The incidence and genetic variability of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *J. Infect. Dis.* **176**:1374–1378.
24. Vinje, J., and M. Koopmans. 1996. Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *J. Infect. Dis.* **174**:610–615.
25. Vinje, J., and M. P. Koopmans. 2000. Simultaneous detection and genotyping of “Norwalk-like viruses” by oligonucleotide array in a reverse line blot hybridization format. *J. Clin. Microbiol.* **38**:2595–2601.
26. Wyatt, R. G., R. Dolin, N. R. Blacklow, H. L. DuPont, R. F. Buscho, T. S. Thornhill, A. Z. Kapikian, and R. M. Chanock. 1974. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. *J. Infect. Dis.* **129**:709–714.